# Effect of routine dentistry on faecal fibre length in Donkeys

Claire Johnson[1], Jane Williams[1]*, and Chelsie Phillips[1]

[1]University Centre Hartpury, Gloucester, Gloucestershire, UK, GL19 3BE.

*Corresponding author: jane.williams@hartpury.ac.uk; 0044 1452702640.

## Abstract

Many donkeys are kept as companions in the UK and are not ridden or work, therefore dental pain can often go unnoticed by owners. Donkeys suffer from an increased frequency of dental pathology compared to horses and require regular dental treatment (rasping) to optimise their welfare. Faecal fibre length (FFL) has been suggested as a non-invasive method to assess when *Equidae* require dental treatment. This study aimed to identify FFL pre-rasping in donkeys requiring dental treatment and to evaluate how this changed over a 6-week period post-rasping.

Twenty adult donkeys of mixed sex and age, and subject to analogous management regimes were selected from the Donkey Sanctuary. Faecal samples were taken for FFL analysis pre-rasping (week 0) and post-rasping (weeks 1, 2, 3 and 6). Mean FFL, determined via laboratory analysis, was recorded for each donkey and the cohort each week. Repeated measures ANOVA with post-hoc Bonferroni analyses and a Bonferroni adjustment ($P \leq 0.01$) examined if differences occurred in FFL between weeks.

The cohort's mean FFL was higher pre-rasping than for all weeks examined post-rasping. Significant reductions in mean FFL for the cohort were reported pre- and post-rasping for week 0 to weeks 1, 2, 3 and 6, weeks 1 and 3, 1 and 6, weeks 2 and 3, and week 2 and 6 ($P<0.0001$). Pre-rasping FFLs >3.3mm were associated with the presence of dental elongations in adult, companion donkeys. This suggest that FFL measurement is a useful non-invasive tool that could be used to assess the dental health of donkeys.

Key words: equine, rasping; prophylactic dentistry; welfare; dental pathologies

## Highlights:

1. Donkeys experience a higher incidence of dental pathologies than horses.
2. Dental pain can be hard to diagnose in unridden companion donkeys.
3. FFL>3.3mm were associated with dental pathology in the donkeys examined.
4. FFL reduced after rasping for the 6 weeks examined.
5. FFL could be used as a non-invasive indicator of dental pathology in donkeys.

## 1.0 Introduction

Modern management regimens [1] and diets of domesticated *Equidae* often restrict access to forage and instead contain high concentrate rations [2]. These diets require reduced attrition and do not cause sufficient wear of the occlusal surfaces needed to maintain hypsodont dentition [3,4]. Subsequently, a higher prevalence of dental abnormalities is reported in managed *Equidae* compared to their free-living peers [5,6]. Domesticated horses and donkeys therefore require regular routine dental treatment (rasping) to facilitate functional mastication and digestion [7,8].

There are approximately 44 million donkeys worldwide [9] the majority of which are working animals [10]. In the UK, donkeys are often kept as companion animals (not ridden), which can result in dental pain not being identified by their keepers and donkeys receiving minimal or no regular dental treatment [11]. Dental pathologies are the second most common clinical condition reported in the domestic donkey [12] and have been widely associated with impaction colic cases [13,14]. Dental pathologies therefore represent a potential welfare issue in the donkey.

To date, the majority of dental care protocols used in the donkey have been adapted from those used in the horse [15]. Yet the assumption that the donkey and the horse are identical is an incorrect with differences between digestive physiology and dental anatomy reported [16, 17]. Both species possess hypsodont dentition, with an annual eruption rate of 2-3mm reported [18,19]. Donkeys possess between 36- 44 teeth dependent upon age, sex and presence of non-functional wolf teeth [17], with the average adult animal presenting with 36 permanent teeth [8]. Donkeys have a greater degree of anisognathia than horses, 27% compared to 24% respectively [17] and a wider range of occlusal angles than the horse [20]. Changes to the masticatory cycle due to either discomfort or an inappropriate diet can produce a more pronounced vertical masticatory pattern resulting in increased occlusal surface angulation [14]. Therefore, the normal cheek teeth angulation and anisognathia found in donkeys, combined with the impact of modern management regimes, predispose them to develop a higher incidence of dental pathologies than the horse [15].

Faecal fibre length (FFL) can be used as an indicator of oral health and masticatory efficiency in *Equidae* [21, 22] and could therefore be used to assess dental health status in donkeys. FFL >3.6mm have been proposed as an indicator of the presence of dental abnormalities in horses [18, 23]. Research in horses suggests that FFL does not significantly change after dental treatment [24, 25]. However these studies used a technique (rubber ball to encourage fibre separation, followed by dry sieving) which could cause excessive attrition of faecal fibres producing measurements which are not representative of true FFL [22, 26]. The validation of FFL as an indicator of masticatory efficiency and digestion in the donkey could provide a monitoring tool informing frequency of routine rasping aiding in the maintenance of welfare in donkeys. Therefore, this study aimed to identify FFL in donkeys requiring dental treatment and to evaluate the effect of routine dental treatment on FFL in companion donkeys over a six-week period. It was hypothesised that a reduction in FFL would occur after rasping.

## 2.0 Materials and Methods

Twenty donkeys of mixed sex (16 Jacks; 4 Jennys) and age (7.6±2.8 years), subject to the same management practices (group housed in a barn with turnout) and diet (haylage twice per day and *ad libitum* oat straw), resident at The Donkey Sanctuary, Woods Farm, Devon, UK were selected for inclusion in the study. All donkeys required routine dental treatment, as part of their ongoing, yearly health care. The study was authorised by the site manager and the management team. All procedures, including dental examinations and treatments were approved as adhering to animal welfare guidelines by the University of the West of England (Hartpury) Ethics Committee and were performed by a qualified equine dental technician (EDT) adhering to British Equine Veterinary Association (BEVA) guidelines [27]. Data collection took place from mid-October to the end of November 2013.

### *2.1 Faecal sampling protocol*

An initial faecal sample was collected prior to any dental examination or treatment: week 0. Individual donkeys were separated from the herd, but they were still in visual contact with the rest of the herd to prevent putting them under undue stress, until they defecated. Faecal samples were then collected from the naturally dropped faecal matter, fifty grams were weighed using digital scales and placed into sealed plastic bags and frozen on the day of collection at -18$^{o}$C, monitored using a digital thermometer. Each bag was labelled with the sample number and a letter which represented the individual donkey. Once a sample had been successfully collected, the donkey was moved back into the barn to prevent re-collection or sampling errors. The yard where the donkeys were held was cleared of any existing faeces prior to and during sample collection to avoid misidentification of the donkey the sample came from. Faecal sample collection was repeated post-dental treatment for weeks 1, 2, 3 and 6 using the same procedure.

## 2.2 *Dental treatment*

Dental examination and treatment was performed over two days after the first (week 0) faecal samples had been collected. All donkeys were treated by the same BEVA qualified EDT who was a member of the British Association of Equine Dental Technicians.. The onsite veterinarian assessed the donkeys and declared them fit to receive treatment and free from any pre-existing clinical conditions other than dental elongations that could be corrected by rasping accompanied by no further pathologies. The 20 donkeys were held in their normal yard whilst receiving dental treatment to minimise stress.

A full oral examination was performed, visualising all dental surfaces/structures and assessing all oral tissues. Donkey age, sex and dental diagnoses data were transcribed directly to a dental chart; dental disorders noted included sharp enamel points, focal overgrowths, shear mouth, step mouth, wave mouth, accentuated transverse ridges and diastema. Routine dental treatment (rasping) was undertaken to reduce overgrowths, remove sharp enamel points, increase lateral excursion, restore balance of the arcades and establish correct occlusal angles in accordance with BEVA guidelines (2009).

## 2.3 *Laboratory analysis of faecal fibre length*

Prior to laboratory analysis, the sampling period individual samples came from was blinded from the experimenter to prevent bias. Faecal samples were defrosted at room temperature (18-24°C) until the sample reached 4°C. Five grams of faecal matter, taken from multiple sections of the larger 50g sample to ensure a representative selection of fibre lengths, was weighed using digital scales. Each 5g sample was added to a glass beaker filled with 500ml of distilled water. The mixture was gently stirred to separate fibres from unwanted sediment. The mixture was then poured through a 0.5mm sieve to eliminate all fibres under 0.5mm from analysis. The remaining fibre mass was collected and gently spread over a foil square, labelled in indelible marker with the sample's identification letter. All 20 samples were placed in the oven at 150°C for 2 hours and once dried each sample was gently sieved through a 1cm sieve, using a soft bristle brush to encourage fibre separation whilst attempting to prevent attrition to the fibre length during the process. The separated dry fibres were re-sieved evenly over a 616 squared grid, sub-divided into four labelled quadrants: A, B, C and D, each of which was subdivided into 154 squares. One square from the 154 present in each quadrant was randomly selected for analysis (e.g. Quadrant A, square 101). Ten faecal fibres were measured from each of the four squares selected, providing a total of forty faecal fibres for each individual sample. Fibres were removed from the grid using tweezers, placed on a separated white surface and were individually measured using Mitutoyo Absolute Digimatic Digital Vernier Callipers (Mitutoyo part number: 500 196-20, model: 500 196-20, accuracy ±0.01mm). The mean, standard deviation, upper and lower and inter-quartile ranges were calculated for FFL of each sample using Microsoft Excel™ Version 2010 prior to statistical analysis. The FFL analysis procedure was repeated for each individual sample for weeks 0, 1, 2, 3 and 6.

### *2.4 Statistical Analysis*

Data were analysed using Statistics Package for Social Scientists (SPSS, Version 20). Data were parametric however whilst Pillaus Trace confirmed a highly significant difference in mean FFL it could not provide specificity (P=0.0001) and Mauchley's test indicated that the assumption of sphericity within the data had been violated (P=0.002). Therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (Ɛ=0.57) and one-tailed Repeated Measures ANOVA with a Greenhouse-Geisser correction was applied to determine if significant differences were present in mean FFL across the cohort [28]. Post hoc Bonferroni analyses were conducted with a Bonferroni correction applied, to adjust for repeated measures, resulting in a revised significance level of P≤0.01. These tests were performed to determine where statistical differences occurred in FFL between the data collection weeks for the entirety of the study.

## 3.0 Results

The cohort's mean FFL pre-rasping was higher than all weeks examined post-rasping (Table 1). The majority of subjects recorded higher FFL (90%) pre-dental treatment compared with their FFL recorded post-dental treatment; the magnitude of FFL changes varied between individual donkeys as well as within the weeks evaluated (Table 2).

Significant changes in mean FFL (decreases) were found across the study period (P<0.0001), however after subsequent post-hoc analysis and Bonferroni adjustment for repeated measures, this pattern was not repeated consistently for the entirety of the study period. Significant reductions in mean FFL for the cohort were reported pre- and post-dentistry for week 0 to weeks 1, 2, 3 and 6 (P=0.0001) with further reductions reported between weeks 1 and 3, 1 and 6, weeks 2 and 3, and week 2 and 6 (P=0.0001). No significant changes in FFL length occurred between weeks 1 and 2, or between weeks 3 and 6 (P>0.05).

Table 1: Faecal fibre lengths in millimetres (to 2 decimal places) across the cohort for the study period.

| Faecal Fibre length (mm) | Pre-dentistry (week 0) | Post-dentistry (week 1) | Post-dentistry (week 2) | Post-dentistry (week 3) | Post-dentistry (week 6) |
|---|---|---|---|---|---|
| **Mean** | 4.37 | 3.03 | 2.80 | 1.95 | 1.93 |
| **Standard deviation** | 0.65 | 0.40 | 0.25 | 0.27 | 0.30 |
| **Minimum** | 3.32 | 2.50 | 2.32 | 1.46 | 1.35 |
| **Lower quartile** | 4.02 | 2.60 | 2.66 | 1.80 | 1.74 |
| **Median** | 4.27 | 3.05 | 2.77 | 1.97 | 1.89 |
| **Upper quartile** | 4.79 | 3.41 | 2.98 | 2.13 | 2.16 |
| **Maximum** | 5.55 | 3.81 | 3.25 | 2.47 | 2.43 |

Table 2: Individual faecal fibre length across the six weeks investigated in millimetres to 2 decimal places

| Donkey ID | Faecal fibre length (FFL) in millimetres (mm) post routine dental treatment | | | | |
|---|---|---|---|---|---|
| | **Week 0** | **Week 1** | **Week 2** | **Week 3** | **Week 6** |
| 1 | 4.01 | 2.52 | 2.32 | 1.80 | 1.86 |
| 2 | 4.82 | 2.93 | 2.54 | 1.96 | 2.13 |
| 3 | 4.51 | 2.59 | 2.98 | 1.80 | 2.41 |
| 4 | 4.93 | 2.56 | 2.93 | 2.24 | 2.23 |
| 5 | 4.11 | 2.66 | 2.90 | 1.63 | 1.85 |
| 6 | 5.46 | 2.60 | 3.20 | 1.46 | 2.33 |
| 7 | 4.62 | 2.50 | 2.64 | 1.51 | 2.31 |
| 8 | 4.29 | 2.91 | 2.43 | 1.89 | 1.75 |
| 9 | 5.34 | 3.19 | 2.86 | 2.40 | 1.66 |
| 10 | 3.32 | 3.47 | 2.51 | 1.98 | 1.63 |
| 11 | 3.58 | 3.01 | 2.77 | 2.05 | 1.91 |
| 12 | 4.65 | 3.26 | 2.93 | 2.18 | 1.94 |
| 13 | 3.38 | 3.46 | 2.70 | 1.66 | 1.75 |
| 14 | 4.04 | 3.50 | 2.76 | 1.82 | 1.35 |

| | | | | | |
|---|---|---|---|---|---|
| 15 | 4.20 | 2.61 | 3.25 | 2.12 | 1.65 |
| 16 | 4.71 | 3.81 | 2.74 | 2.47 | 1.70 |
| 17 | 4.04 | 3.15 | 2.75 | 1.99 | 1.78 |
| 18 | 4.24 | 3.08 | 3.03 | 2.01 | 2.08 |
| 19 | 3.64 | 3.22 | 3.09 | 1.88 | 1.93 |
| 20 | 5.55 | 3.56 | 2.73 | 2.13 | 2.43 |
| **Cohort** | | | | | |
| Mean | 4.37 | 3.03 | 2.80 | 1.95 | 1.93 |
| Standard deviation | 0.65 | 0.40 | 0.25 | 0.27 | 0.30 |

## 4.0 Discussion

At the start of the study, the majority of donkeys (90%) exceeded a FFL of >3.6mm the length proposed to indicate the presence of dental abnormalities in horses [26, 29]. The presence of dental pathologies were confirmed in these donkeys by EDT examination. However, EDT examination confirmed a further two donkeys, who returned FFL <3.60mm (3.32 and 3.38mm respectively), required dental treatment suggesting that the FFL level that is consistent with the presence of dental abnormalities may be shorter in donkeys than that proposed in the horse, however more research is required before this is confirmed. By week 3, the FFL for all donkeys appeared to stabilise at lengths <2.50mm. Our results suggest that FFL measurement is a useful non-invasive tool that could be used to assess the dental health of donkeys, with FFL >3.30mm indicating the presence of dental elongation in adult donkeys.

The FFL length of the majority of donkeys (90%) reduced a week after rasping, but 5 (25%) still presented with a FFL >3.3mm. However by week 2, all donkeys' FFL were >3.3mm and further reductions in FFL occurred up to week 6. Routine rasping removes dental pathologies, thus reducing restriction to occlusal contact allowing full excursion and improved attrition, facilitating more efficient mastication [30]. The variation reported here suggests that the more efficient attrition which occurs post rasping, generates a reduction in faecal particle size [31, 32]. Kinematic and electromyographic evaluation of how the mastication cycle in horses changes post-rasping, suggests that the first week after dental treatment (rasping) represents a period where fluctuations occurs in the mastication cycle demonstrated by changes in lateral excursion and the power stroke [31] and masseter and temporalis muscle workloads [32]. This adaptation could explain why there appears to be a transition period of 1 to 2 weeks for some donkeys before FFL reduces below 3mm. Interestingly, donkeys that recorded FFL >3.3mm presented with more severe dental elongations pre-rasping than their peers; therefore the rate of FFL reduction post-rasping, may also be influenced by the incidence and severity of dental pathologies present in the subject.

### *4.1 Limitations and further research*

The results of this preliminary study are promising; however, further work incorporating larger numbers of donkeys to confirm the results found here and to establish a standardised FFL indicator of dental pathologies in donkeys is required. The current sample considered adult, companion donkeys, therefore we would advocate repeating the study in working donkeys and across wider age ranges to evaluate if differences in FFL present between adult and geriatric samples.

## 5.0 Conclusion

Routine dental treatment resulted in significant reductions in FFL in donkeys, which suggests that rasping has improved the efficiency of mastication. Our results suggest that faecal fibre lengths of <3.3mm can be used as an indicator of the presence of dental pathologies in companion, adult donkeys. If a standardised FFL length can signpost the presence of dental pathologies, the measure has the potential to be implemented as a standard welfare indicator particularly for working donkeys globally.


## Acknowledgements

We would like to thank the Donkey Sanctuary for their support and providing access to the donkeys during this study.

## Conflict of interest

No conflicts of interest apply to this work.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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